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Neisseria gonorrhoeae: Issues in susceptibility testing and resistance

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The treatment and control of gonorrhea has been complicated by the development of antimicrobial resistance in *Neisseria gonorrhoeae* (GC), changes in the availability of antimicrobials recommended for treatment, and decreases in the availability of isolates for susceptibility testing as culture methods are replaced by nucleic acid amplification tests (NAAT).

The current Centers for Disease Control and Prevention (CDC) recommended primary therapies for uncomplicated gonococcal infections of the cervix, urethra, and rectum include ceftriaxone, cefixime, or a fluoroquinolone (ciprofloxacin, levofloxacin, or ofloxacin) (3). In July of 2002, Wyeth Pharmaceuticals discontinued manufacturing cefixime, the only recommended oral cephalosporin (8). CDC has not recommended another oral cephalosporin to replace cefixime, but studies are underway to determine the efficacy of other agents such as cefpodoxime or cefuroxime axetil. The Food and Drug Administration (FDA) has approved cefpodoxime and cefuroxime axetil for treatment of uncomplicated gonnococcal infections.

In November 2003, the Michigan Association of Local Public Health Physicians Forum recommended that local health departments use a single 400 mg dose of cefpodoxime proxetil for uncomplicated gonorrhea treatment when an oral cephalosporin is indicated. The MDCH is securing a supply of cefpodoxime and will make it available for use in local health department STD Clinics sometime in the first quarter of 2004. Beginning January 1, 2004, MDCH added cefpodoxime to and deleted cefixime from its routine susceptibility testing panel for GC. The other antimicrobials tested are: ceftriaxone, ciprofloxacin, spectinomycin, and tetracycline.

After the appearance of penicillin and tetracycline resistance in GC in the 1970s, CDC established the Gonnococcal Isolate Surveillance Project (GISP) in 1986 to monitor changes in antimicrobial susceptibility patterns in GC. A limited number of isolates for testing are collected on a monthly basis from participating STD clinics (27 cities represented in 2002) across the United States (2). Between 1988 and 1994, five spectinomycin-resistant isolates have been reported by GISP (1). While no ceftriaxone- or cefixime-resistant isolates have been found, several isolates with decreased susceptibility to these agents have been described. Four isolates with decreased susceptibility (MIC = 0.5 mcg/ml) to ceftriaxone were found between 1987 and 1997 (1). There have been 45 isolates with decreased susceptibility to cefixime (MICs from 0.5-2.0 mcg/ml) (2). No isolates resistant to spectinomycin or with decreased susceptibility to the cephalosporins have been found in Michigan.

In the early 1990s, reports of fluoroquinolone-resistant GC (QRNG) began to appear worldwide and resistant strains became well established in several areas (e.g. Thailand, Hong Kong, Japan,

and the Philippines). Sporadic reports of QRNG in the United States were generally associated with travel to Asia. Isolation of QRNG in Hawaii steadily increased from 1.4% (4 of 290) in 1997 to 20.3% (16/79) in 2001 (6,7). In 2000, California reported an increase in QRNG in San Francisco, San Diego, and Orange County. In 2001, 2.5% (33 of 1,311) of isolates tested were QRNG with 3.4%, 3.0%, 3.3%, and 2.4% of isolates from San Francisco, Long Beach, Orange County, and San Diego, respectively, being resistant (7). In 2002, the prevalence of QRNG continued to increase in California with 6.7%, 7.2%, 11.4%, and 16.5% of isolates tested from San Francisco, Long Beach, Orange County, and San Diego, respectively, being resistant (2). As a result of the increasing prevalence of QRNG, the Hawaii Department of Health and California Department of Health Services have recommended that clinicians avoid the use of fluoroquinolones to treat gonorrhea (3). CDC also recommended that fluoroquinolones not be used to treat GC acquired in Asia, the Pacific Islands, Hawaii, California or other areas with an increased prevalence of QRNG (7). The prevalence of QRNG in other parts of the world are: Australia, 8.1%; China, 92.5%; Japan, 73.4%; Korea, 63.3%; Philippines, 57.5%; Singapore, 46.5%; Vietnam, 46.0% (5); Israel, 61% (4); and Canada, 2.1% (9).

In addition to Hawaii and California, QRNG isolates have been reported from Anchorage, Atlanta, Miami, New Orleans, Philadelphia, Las Vegas, Portland, Dallas, Cleveland, Denver, Portland, Cincinnati, Chicago, Minneapolis, Seattle, New York City, and Phoenix. Resistant isolates were also reported from Illinois, New Hampshire, Wisconsin, Massachusetts, New Jersey, Michigan, and Utah (1,2). Preliminary GISP data for 2003 shows a significant increase in QRNG in Seattle, Massachusetts, New York City, Michigan, Phoenix, Minneapolis, Chicago, Las Vegas, and Portland (CDC unpublished).

Michigan reported one QRNG case in 2001 and one in 2002, both of which were connected to travel in Asia. In the first three months of 2003, MDCH identified four cases of gonorrhea in Ingham County caused by QRNG. None of the cases reported any recent travel and no travel history was documented in their contacts. After consultation with CDC, MDCH advised clinicians that all gonorrhea cases in Ingham and the adjoining counties of Clinton, Eaton, Jackson, Livingston, and Shiawassee be treated with a non-quinolone regimen. Four additional cases of QRNG were identified in Ingham County during the remainder of 2003. In June of 2003, three cases of QRNG were identified in Kent County. Two of these cases were men with a history of sexual contact with other men (MSM) and a history of multiple sexual partners. The Kent County Health Department recommended that quinolones could be selectively used in Kent County, but that clinicians should aware of the potential for QRNG infections. MDCH reported one ciprofloxacin-intermediate and 17 ciprofloxacin-resistant isolates from 15 patients in 2003. QRNG isolates came from Ingham (9), Kent (4), Washtenaw (3), Genesee (1), and Oakland (1) counties.

As the prevalence of QRNG began increasing, CDC recommended that state health departments monitor local antimicrobial susceptibility patterns to guide local treatment recommendations (7). To augment routine susceptibility studies performed at MDCH, a special surveillance project collecting GC isolates from five clinical laboratories across the state was started in July 2002. Table 1 shows a summary of GC susceptibility testing results from 2001 – 2003. Data from the surveillance project suggests that resistant isolates are localized rather than widespread. The Detroit City Health Department joined the GISP project in 2003, but isolates collected from that site failed to document any QRNG isolates. This suggests that surveillance isolates should be collected from multiple geographically diverse sites to detect emerging resistance.

With the increasing popularity of NAATs for GC and chlamydia, many clinical laboratories have seen dramatic decreases in the number of GC cultures performed. While NAATs offer several advantages over routine culture, they fail to provide an organism for susceptibility testing. MDCH will continue to provide GC susceptibility testing to monitor the emergence of resistance when isolates are available. To improve surveillance efforts, MDCH recommends that clinicians culture patients that have failed treatment for gonorrhea. Positive cultures should be sent to MDCH for susceptibility testing. Clinical laboratories can also assist by submitting isolates from patients that are repeatedly culture positive.

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Table 1. Susceptibility testing results for *Neisseria gonorrhoeae* isolates Michigan 2002-2003

Antimicrobial	Year	# tested	# susceptible	# intermediate	# resistant
Cefixime	2001	59	59	0	0
	2002	187	187	0	0
	2003	582	582	0	0
Ceftriaxone	2001	59	59	0	0
	2002	187	187	0	0
	2003	582	582	0	0
Ciprofloxacin	2001	59	58	0	1
	2002	187	186	0	1
	2003	582	564	1	17
Spectinomycin	2002	160	160	0	0
	2003	582	582	0	0
Tetracycline	2002	157	58	93	6
	2003	582	169	370	43

"Fun Fungi…"
Will Return
in the
Spring Issue
of *LabLink*

Laboratory Director Appointed to National Board

Susan L. Shiflett

Frances Pouch Downes, Dr. P.H. has been appointed as a member of the Centers for Disease Control and Prevention (CDC)'s Board of Scientific Counselors. The term begins immediately and will expire on September 30, 2007.

The Board of Scientific Counselors is comprised of 24 national experts on epidemiology and disease control. The board is responsible for evaluating strategic plans to combat infectious diseases in the United States. The board recommends specific plans of action to the CDC.

"We are privileged to have such a consummate public servant and nationally renowned disease expert in charge of our public health laboratory operation in Michigan," said Janet Olszewski, Director of the Michigan Department of Community Health. "Dr. Downes brings a wide range of experiences to the table for the CDC, and the knowledge she will gain from her service will ultimately benefit the citizens of Michigan."

Downes holds masters and doctorate degrees in laboratory practice from the University of North Carolina at Chapel Hill. She was a pre-doctoral fellow at the CDC from 1985 until 1987.

Downes has served as the administrator for the Bureau of Laboratories since 1999. Prior to this post, she served as the Director of the Infectious Disease Division (1997-1999) and the managed care coordinator for the laboratory (1995-1997).

Downes serves on the U.S. Department of Agriculture's National Advisory Committee for the Microbiological Standards for Foods and was the co-editor of the *Compendium of Methods for the Microbiological Examination of Foods (4th Edition)* for the American Public Health Association. She is an adjunct professor in the Medical Technology and International Health Program at Michigan State University.

Currently Downes is a team leader for the Association of Public Health Laboratories' Botswana Global AIDS Laboratory Project. The project is designed to create public health laboratory capacity and quality assurance for the testing and treatment of human immunodeficiency virus (HIV) in that country.

The Bureau of Laboratories would like to congratulate Dr. Downes on her appointment.

Enterohemorrhagic *Escherichia coli*: Recent experience in Michigan

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Shannon D. Manning, Ph.D., M.P.H.

Enterohemorrhagic *E. coli* (EHEC) causes bloody diarrhea, hemorrhagic colitis, and may progress to hemolytic uremic syndrome (HUS). In 1993, EHEC was identified as the causative agent in a large, multi-state outbreak involving ground beef, and continues to be linked to outbreaks involving both meat and non-meat products (e.g., lettuce, cider). EHEC strains produce the shiga-like toxin (STX), a potent toxin that facilitates disease development. In the United States, most EHEC strains are serotype O157:H7, while non-O157:H7 strains appear to be less common. The actual prevalence of all EHEC serotypes is not well documented and is likely to be underestimated. In Europe up to 70% of EHEC infections are caused by non-O157 serotypes; up to 20 different non-O157 serotypes have been identified to date.

In the laboratory, detection of O157 strains usually results from subculturing stools on sorbitol-MacConkey agar (SMAC). EHEC

O157 typically does not ferment sorbitol and thus does not produce red colonies on SMAC. In contrast, detection of non-O157 serotypes by culture is problematic because these strains typically ferment sorbitol, thereby producing red colonies on SMAC. Thus, most non-O157 EHEC colonies appear like the non-pathogenic *E. coli* strains commonly found in the gut flora. Recognition of the non-O157 serotypes often depends on the use of non-culture based techniques, such as an enzyme immunoassay (EIA) or PCR, that detect the presence of the toxin or the toxin genes, respectively, in both O157 and non-O157 strains.

In order to assess the crude prevalence of both serotypes in Michigan, EHEC isolates confirmed at MDCH between 2002 and 2003 were analyzed. Of the 201 isolates, 188 (94%) EHEC were O157. The remaining 13 strains were non-O157 groups. Most of the sorbitol-negative O157 strains were identified by clinical laboratories using SMAC and were submitted to MDCH for confirmation.

The non-O157 strains were collected by MDCH in one the following ways: 1) they were isolated from bloody stool specimens submitted to MDCH for EIA testing; 2) they were isolated from the stool of patients with documented HUS that was submitted to MDCH for EIA testing; or 3) they were isolated from stools that yielded a positive EIA result at a clinical laboratory but no sorbitol-negative colonies were recovered from culture at the clinical laboratory.

In 1994 CDC recommended that all stools be cultured for *E. coli* O157:H7 regardless of season, patient age or presence of blood in the stool. A survey of Michigan laboratories conducted in 2001, however, found that 49% do not use SMAC at all. Consequently, the prevalence of O157:H7 strains also may be underestimated in Michigan.

Despite efforts to reduce food contamination, EHEC outbreaks are still common. Citizens of Michigan, like those of other states and countries, continue to suffer consequences ranging from self-limiting diarrheal illness to death. While the incidence of EHEC infections may not be high when compared to infections caused by *Salmonella* spp., the clinical disease associated with EHEC is often far more severe. If SMAC is used to screen for the presence of O157 strains, then microbiologists should note the patient diagnosis, if available, and the characteristics of stool submitted for culture. In patients presenting with bloody stools or with a history of hemorrhagic colitis, laboratories should consider the presence of non-O157 serotypes when SMAC culture fails to produce typical colonies. If an EIA is used to screen for EHEC and culture of an EIA-positive stool fails to yield sorbitol-negative colonies on SMAC, non-O157 strains may be responsible for the infection. In either case, submission of the suspect stool or a swab of the confluent growth of the SMAC plate to MDCH is recommended.

Packaging and Shipping Training Coming to a Lab Near You!

MDCH Bureau of Laboratories is now offering a one-day "Packaging and Shipping Infectious Substances and Diagnostic Specimens" program. This program will provide a comprehensive overview of federal and international regulations. Successful completion of this training meets minimum regulatory certification requirements.

For further information or to request this training opportunity, please contact:

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The Bureau of Laboratories Welcomes Jonathan Duczkowski

Jeff Massey, Dr. P.H.
Molecular Biology Section

The Molecular Biology Section announces the arrival of Jonathan Duczkowski as an Emerging Infectious Disease (EID) training fellow. The EID training fellowship is a 12 month program sponsored by the Association of Public Health Laboratories (APHL), in collaboration with the Centers for Disease Control and Prevention (CDC). It is intended to prepare laboratory scientists for careers in public health and environmental health laboratory practice. Two programs are available: a 12 month training fellowship for individuals with a B.S. or M.S. degree and a two year post-doc research fellowship for individuals with a doctoral degree.

Duczkowski arrived at MDCH in September 2003. He graduated from Michigan State University (MSU) in May 2003 with dual Bachelor of Science degrees in Medical Technology and Zoology. He has received a Certificate in Molecular Diagnostics through MSU.

In addition to his interest in molecular diagnostics and public health, Jon claims to have additional talents "In addition to my extensive readings in history, I have spent much time working on cars and trucks; I suspect that I am one of a very small number of applicants who have a history of rebuilding engines."

When not working on engines, Duczkowski is making significant contributions to the Molecular Biology Section. In four months he has already demonstrated the feasibility of recovering and amplifying DNA stored on dried blood spots (DBS) stored for up to 21 years. These findings have important implications in future testing of DBS for genetic disease. Other projects that he will be tackling include implementation of a real-time PCR procedure for detection of staphylococcal detection in outbreaks of foodborne illness and development of a real-time PCR procedure to detect *Borrelia burgdorferi*, the causative agent of Lyme disease.

More information on this program may be found at http://www.aphl.org .

New Library Items

Susan L. Shiflett

The MDCH Laboratory Library has four new videos and two new CD-ROMs available to borrow.

Videos include "Update on Rapid Testing for HIV" a CDC 4/24/03 satellite broadcast, "Assessing Risks of Toxic Chemicals" form the Howard Hughes Medical Institute, "Chemical Terrorism Preparedness: The Basics," sponsored by the NLTN and "Bioterrorism and Zoonosis" a Center for Public Health Preparedness Grand Rounds satellite broadcast. The new CDs are the "DPDx, Laboratory Identification of Parasites of Public Health Importance" from CDC and "The Primate Malarias" also from CDC.

To request an MDCH library item, or to get a complete list of items available, phone 517-335-9972 or email shifletts@michigan.gov. For more training materials visit the National Laboratory Training Network library at www.phppo.cdc.gov/libnltn .

Regional Laboratory Wet Mount Proficiency

William S. Sottile, Ph.D. ABMM
Michigan Regional Laboratory System

The Clinical Laboratory Improvement Amendment of 1988 (CLIA'88) requires any laboratory performing wet mount microscopy for the diagnosis of vulvo-vaginitis have either a Provider Performed Microscopy (PPM) CLIA certificate or a moderately complex CLIA certificate. The Michigan Regional Laboratory Committee covers Nurse Practitioners in the Regional Lab System under a moderately complex CLIA certificate allowing more flexibility in the tests performed. Both certificates require demonstrated competency and proficiency in test performance.

There are a few external proficiency contractors, known as 'deemed providers' that have commercial surveys for provider-performed microscopy. Those services available evaluate samples for tests other than vaginal swab specimens. The commercial survey usually provides only a single slide (35mm Kodachrome transparency) of each specimen for the participant to evaluate. A single challenge might contain a slide for urinalysis, a slide for fern tests, a slide for pinworm detection and a slide of vaginal discharge. CLIA requires that each site submitting proficiency data maintain a score of at least 80% on consecutive surveys or the procedure must be suspended. If the practitioner only performs wet mounts for vaginal discharge, their whole score would be based on only one slide. Even if there are two or three slides per year, a single error would result in suspension of the procedure.

For the past three years, the Michigan Regional Laboratory System has used printed photomicrographs as tools to evaluate proficiency. Each challenge contains three photomicrographs, containing one to three different elements to be identified. In some cases there are three to nine items to be identified, resulting in a wider range of material for the microscopist to evaluate. Since proficiency is based on identification of five or six different objects, missing one does not necessarily result in failure.

Feed back from participants indicates that some would rather have an actual sample to examine. Practitioners would like to be provided the results of pH and 'whiff tests', and the client's general symptoms and history. It is impossible at this point to distribute these materials because they are unstable and would not yield the sane results as freshly stained specimens routinely used in wet mount examination. An actual diagnosis is not sought. The objective of the wet mount proficiency challenge is to present common microscopic objects for identification.

In order to assist practitioners in evaluating the wet mount proficiency challenges as presented, MDCH has included copies on the Regional Laboratory website. Navigate the computer browser to http://www.michigan.gov/mdchlab and select the following in order:

"Regional Labs"
"Internal Proficiency for Wet Mount Microscopy

The original challenge and the critique are posted. The files are in Adobe Acrobat format and can be downloaded and printed out for training and reference. Each micrograph is included for those who might wish to enlarge the object(s) for a better view, although the result is much larger than would be observed at 1000x on a standard microscope. The standard laboratory procedure, "*Wet Mount Microscopy of Vaginal Discharge Fluid*" has been updated to include micrographs to assist in interpretation and is available on the web site.

Quirky Bugs…
Coryneforms in Urine

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Reference Bacteriology Unit

Two urine culture isolates of a coryneform bacteria were recently submitted to the Reference Bacteriology Unit at MDCH that underscore some important considerations when dealing with such organisms.

For many years, coryneform bacteria from urine samples were often considered contaminants, and most clinical laboratories pursued no further testing. Given the fact that many of these organisms are part of the normal flora of the skin and mucous membranes in humans, this approach certainly seemed rational. However, it is now widely accepted that the pathogenic potential of coryneform bacteria other than *C. diptheriae* is greatly underestimated, as disease associations are being demonstrated, for example *Corynebacterium urealyticum*, an isolate often associated with urinary tract infections.

Identification to the species level of coryneform bacteria isolated from urine specimens should consider assessment of the colony count. If the culture is pure and the colony count is >10,000 CFU/ml, or if it is the predominant organism and the colony count is >100,000 CFU/ml, identification to the species level is usually warranted. Other factors to weigh in the determination of an isolate's clinical significance is whether multiple specimens are positive for the same coryneform, if the organisms are observed in a direct Gram stain, a strong leukocyte esterase reaction, and if any other organism recovered from the same sample are of low pathogenicity.

The two coryneform isolates submitted to MDCH for identification were from the urine of two elderly female nursing home patients. The initial Gram stain revealed typical club-shaped coryneform gram positive rods. After 48 hours of incubation on 5% sheep blood agar plates, white, glistening convex colonies with entire edges up to 1.5 mm in diameter were observed. Consistency was creamy or slightly sticky. The organisms were catalase positive and non-motile.

A striking feature was the strong urease activity, with positive results within five minutes after inoculation at room temperature on Christensen's urea agar slants. A strong urease activity has been demonstrated as a virulence factor for a variety of genitourinary bacterial pathogens, which may also be the case for these isolates. Considering the urine source and the strong urease activity, one might at first glance think that these isolates were *Corynebacterium urealyticum*, but this proved not to be the case.

These isolates were non-lipophilic, whereas *C. urealyticum* is considered a lipophilic organism. The determination of whether an organism requires lipids for growth is demonstrated by growth on a lipid containing media such as sheep blood agar and either poor or no growth on commercially available chocolate agar, which contains hemin powder instead of lysed erythrocytes. These isolates grew equally well on both 5% sheep blood agar and chocolate agar.

The most unique phenotypic feature of these isolates was the ability to slowly ferment maltose but not glucose, which distinguished them from all other presently defined non-lipophilic fermentative corynebacteria. Other features of these isolates included negative nitrate reduction, negative esculin hydrolysis, negative CAMP reaction, and negative acid production from sucrose, mannitol, and xylose. In addition, these isolates were sensitive to penicillin and grew weakly anaerobically. These last two features further distinguished them from *Corynebacterium urealyticum*, which is a strict aerobe and usually penicillin resistant (although rare penicillin sensitive strains have been described). API Coryne system codes included 0101224, 2001224, and 2101224. Cellular fatty acid analysis was typical for the Genus *Corynebacterium*. These isolates were identified as *Corynebacterium riegelii*.

C. riegelii was first described in 1998 from urine cultures isolated from females with urinary tract infections. Additional isolates have since been recovered from blood cultures. Although it is probably rare, the peculiar characteristic of the slow fermentation of maltose but not glucose should help make *Corynebacterium riegelii* easily recognizable in the routine microbiological laboratory.

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WNV Testing in 2003

Hema Kapoor, MD
Virology Section

In 2002, MDCH continued to accept specimens until December. The testing was extended beyond the traditional arbovirus infection season to detect cases related to blood transfusions or travel to areas where WNV is endemic year round. Out of 147 cerebrospinal fluid (CSF) specimens tested for WNV IgM antibodies in our laboratory from December 2002 through April 2003, three samples showed persistent WNV IgM antibodies. These samples were drawn at 110, 141 and 199 days post-onset of symptoms in patients with meningoencephalitis diagnosis.

In 2003, a total 989 specimens (CSF, n=617 and sera, n=372) were tested, a third of the number received in 2002 for arbovirus testing. The first laboratory confirmed case of WNV in 2003 was reported on September 26, 2003 as opposed to the first confirmed case of the 2002 outbreak, which was reported on August 11. Using the CDC case definition, there were nine confirmed and five probable cases of WNV in Michigan in 2003. Even though 13 of these 14 cases showed signs of meningitis, meningoencephalitis or encephalitis, cerebrospinal fluid (CSF) specimens from these cases showed equivocal results for IgM antibodies for WNV in two samples, equivocal for SLE in one sample and equivocal for both WNV and SLE in nine samples by capture enzyme linked immunoassay (MAC – ELISA) as seen in Table 1.

These findings are different from those seen in 2002. In 2002, with a higher prevalence of WNV, MAC-ELISA testing on CSF specimens from the patients with neurologic symptoms was more diagnostic as compared to 2003. In 2003, almost all the final positive results had to undergo confirmatory testing by Plaque Reduction Neutralization Test (PRNT) as shown in Table 1.

Additionally, 5 CSF specimens were found positive for SLE by the MAC-ELISA test. This may be due to increased surveillance and testing for arboviruses. In five cases (1 CGV and 4 WNV) positive IgG levels in the acute serum sample were the same level of IgG persisting in the convalescent serum sample drawn at least 3 weeks post onset of illness. Case investigation indicated that the on-set of disease determined these cases to be from the 2002 outbreak and they were not counted as 2003 cases.

Table 1

Case #	Specimen type	SLE IgM P/N	WNV IgM P/N	EEE IgM P/N	CGV IgM P/N	PRNT WN	PRNT SLE
1	CSF	EV	EV	N	N	Pos	N
2	CSF & Serum	EV	EV	N	N	S pos	N
3	CSF & Serum	Unint	EV	N	N	S Pos	N
4	Paired sera	NA	NA	NA	NA	NA	NA
5	Ac. Serum	NA	NA	NA	NA	Pos	N
6	CSF & Serum	EV	EV	N	N	S pos	N
7	CSF & Serum	EV	EV	N	N	S pos	N
8	CSF & Serum	EV	EV	N	N	S pos	N
9	CSF & Serum	N	EV	N	N	S pos	N
10	CSF & Serum	EV	N	N	N	S pos	N
11	CSF & Serum	EV	EV	N	N	S pos	N
12	CSF & Serum	EV	EV	N	N	S pos	N
13	CSF & Sera	EV	EV	N	N	S pos	N
14	CSF & Serum	EV	EV	N	N	S pos	N

SLE-St. Louis encephalitis
WNV- West Nile virus
EEE-Eastern Equine encephalitis
CGV-California group virus
PRNT-plaque reduction neutralization test
CSF-cerebrospinal fluid

EV-Equivocal
N-Negative
S-Serum
Pos-positive
Unint-uninterpretable
NA-Not available.

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